

**ASSESSMENT OF FUNCTIONAL STATUS OF
AUTO-TRANSPLANTED PARATHYROID GLANDS
AFTER TOTAL THYROIDECTOMY**

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CERTIFICATE

This is to certify that this dissertation on 'Assessment of functional status of auto-transplanted parathyroid glands after total thyroidectomy' is a bonafide dissertation by Dr. Himagirish. K. Rao conducted in Madras Medical College under the supervision and guidance of and is submitted to The TamilNadu Dr. M. G. R. Medical University, Chennai in partial fulfilment of the requirement for the M.Ch (Endocrine Surgery) degree.

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First described in the Indian rhinoceros in the year 1862 [1], the parathyroid glands are intimately related to the thyroid gland. Normal parathyroid function is integral to the calcium homeostasis in the body. However, thyroid surgery in general and total thyroidectomy in particular is associated with a significant risk of parathyroid injury, post-operative hypoparathyroidism and hypocalcemia. While acute hypocalcemia has various dramatic clinical features ranging from paresthesias to seizures and even death, chronic hypocalcemia can result in crippling complications including heart failure and cardiomyopathy.

Generally, post-operative hypocalcemia and hypo-parathyroidism are transient phenomena which resolve spontaneously over time. Definitive hypo-parathyroidism and chronic hypocalcemia occur as a result of irreversible loss of function of the parathyroids, invariably due to inadvertent removal or ischemic necrosis of the parathyroid glands. So, definitive hypo-parathyroidism is a complication that can be prevented with better intra-operative care.

The best approach to prevent definitive hypo-parathyroidism is to identify the parathyroids and their blood supply during surgery. Should the gland be inadvertently removed or rendered ischemic due to vascular compromise, it can be auto-transplanted to a heterotopic site. The parathyroid tissue has certain unique properties, by virtue of which auto-

transplantation is a viable option in order to prevent chronic hypoparathyroidism and hypocalcemia.

However, the success of this procedure depends upon whether the transplanted tissue is viable and functional. Functionality of the graft can be assessed by confirmation of parathormone (PTH) secretion. This can be determined by estimating serum level of PTH in the effluent from the graft in the draining veins, and by comparison with serum level of PTH in the peripheral circulation.

This study was conducted in the Department of Endocrine Surgery, Madras Medical College with an objective to assess the functionality of the auto-transplanted gland during thyroidectomy and parathyroid auto-transplantation.

2.1 HYPOCALCEMIA AND HYPO-PARATHYROIDISM

The primary objective of this study was to assess the functional status of the auto-transplanted parathyroid gland during total thyroidectomy. However, the focus of this study is post-operative hypo-parathyroidism and hypocalcemia after thyroid surgery. Attempts at resolution are helped no end by a thorough understanding of the problem, including epidemiology, causative factors, clinical presentation and management of post-operative hypocalcemia and hypo-parathyroidism.

2.1.1 Epidemiology

While clinically significant hypocalcemia following thyroid surgery is due to hypoparathyroidism [2-5], it is generally temporary and limited to the first few weeks after surgery. Various researchers have reported that the incidence of transient hypocalcemia following thyroid surgery is 2% - 51% [6-10]. Adverse events tend to be under reported in the literature and so, the true incidence of permanent hypoparathyroidism is difficult to ascertain. However, the incidence of permanent post-

operative hypoparathyroidism complicating thyroid surgery ranges between 0% and 43% [4, 11-13].

It is evident from world literature that transient hypocalcemia is seen so frequently after total thyroidectomy that it is now considered a sequel of total thyroidectomy and is no longer considered a complication.

While symptomatic hypoparathyroidism can be dramatic in presentation, it is clinically occult in the majority of patients with only biochemical evidence of hypocalcemia and sub-normal S. PTH levels.

2.1.2 Etiology: factors that increase the risk of post-operative hypocalcemia

The main cause of hypoparathyroidism following total thyroidectomy is mechanical parathyroid injury or inadvertent parathyroidectomy.

Injudicious use of electro-cautery, especially uni-polar diathermy, will increase the risk of parathyroid injury. Although they may be mechanically intact, the risk for thermal injury and coagulation is increased. So, extreme care is advocated with regards to uni-polar diathermy, once the investing layer of cervical fascia is opened.

Bleeding will muddle the field of surgery and as a result, accurate identification of the vital structures including parathyroid glands is

hindered. So, perfect hemostasis during surgery, especially in the proximity of parathyroid glands, is vital and is best achieved by meticulous dissection and bipolar diathermy, if available.

Magnification of the field with the help of loupes is one method of improving the identification of the glands and their blood supply [14]. However, it takes time to adjust oneself to the usage of loupes during surgery.

In spite of the best and supposedly successful efforts to preserve the anatomical integrity of the parathyroids, hypoparathyroidism can and does occur. A mechanically intact parathyroid gland may be rendered ischemic due to vascular compromise. Although the gland itself and its blood supply may be structurally intact, hypoparathyroidism may occur due to spasm of the vessels.

Research has revealed that patients with normal S. iPTH levels (intact PTH in circulation) at the end of surgery can develop subnormal levels after surgery [15].

In a study by Vidyasagar, et al. [15], the intra-operative and post-operative iPTH levels were estimated and correlated with the number of parathyroid glands which were devascularized or removed during total thyroidectomy. All those patients who had two or more viable and functional glands had normal levels of iPTH, with no significant

difference between the intra-operative iPTH levels among them. In those patients with three devascularized glands, the iPTH levels were subnormal, while iPTH was undetectable after devascularization of four parathyroids. In a similar study, Rafferty, et al. [16] correlated post-op hypocalcemia with number of glands found in the specimen after thyroidectomy. The finding of 3 or more parathyroid glands in the specimen correlated with an incidence of 100% for post-operative hypocalcemia, while the incidence rate was 18% or less when two or fewer parathyroid glands were found on the specimen.

So it follows that at least two viable, functional parathyroid glands are necessary for maintenance of normocalcemia and euparathyroidism post-operatively.

Histopathological examination of the operated specimen confirms inadvertent parathyroidectomy. When evaluated so, the incidence of unintentional parathyroidectomy ranges from 9% to 19% in different series [17, 18]. Inadvertent parathyroidectomy during thyroid surgery is another factor that will influence post-operative hypoparathyroidism.

Recurrent goitre and revision thyroidectomy is also associated with an increased risk of transient and permanent postoperative hypoparathyroidism [17, 21].

Risk of post-operative hypoparathyroidism and hypocalcemia is also increased in thyroid carcinoma and the procedure of lymph node dissection that may be necessitated [2, 21]. Moley and de Benedetti [22] reported that if adequate central node dissection performed, then it is extremely difficult to preserve adequately perfused parathyroid glands in situ, especially the inferior parathyroids.

In addition to anatomical and surgical reasons for postoperative hypoparathyroidism and hypocalcemia, there are other factors which influence the development of post-operative hypoparathyroidism and hypocalcemia.

Graves' disease [17] and thyrotoxicosis in general [19], lead to an increased risk of transient and permanent postoperative hypoparathyroidism. The higher incidence of transient hypocalcemia in thyrotoxic patients is related to the increased skeletal avidity for calcium associated with this pathology [4].

Indeed, there appears to be a correlation between temporary postoperative hypocalcemia and free thyroxine levels [20].

Apart from thyroid surgery, when the parathyroid glands are obviously at risk of injury and necrosis, hypocalcemia has been shown to occur after other surgeries such as herniorrhaphy as well [4]. This decline in serum calcium was seen to occur within 24 hours of surgery. The

change in the levels of calcium and other electrolytes in circulation was found to be very similar to that seen after thyroidectomy.

In the post-operative setting, macrodilution of the intravascular fluid compartment may occur due to excessive replacement of intravenous crystalloids. This may result in hypocalcemia [23]. General anesthesia has been reported as a cause for post-operative hypocalcemia [23].

Calcium homeostasis is influenced by another similar divalent cation, Magnesium. Hypomagnesemia renders the parathyroids less sensitive to falling calcium levels, thus suppressing PTH secretion. In addition, the PTH receptors are also rendered less responsive to PTH. Indeed, hypo-magnesemia has been reported to be an important cause of post-operative hypocalcemia [23]. However, this is difficult to detect and should be suspected in cases of hypocalcemia refractory to intravenous Calcium replacement.

Extensive surgery entails prolonged periods of exposure of the field to low ambient temperatures, causing hypothermia, which may result in hypocalcemia. Hypothermia in the field due to air-conditioning in the operating theatre could be a factor and this can be addressed by periodic irrigations with warm saline. This was evident in a study by Testini, et al. [24], when it was found that the risk of post-operative

hypocalcemia was low when the operative field was irrigated with lukewarm saline from time to time.

So, it is evident that there is a multitude of factors which influence the level of Calcium in the circulation in the post-operative period.

2.1.3 Hypocalcemia: Definition and Clinical presentation

S. Calcium is measured both in mmol/l and mg/dl. 1 mmol/l is equivalent to 0.25 mg/dl. Eucalcemia or normocalcemia refers to S. Calcium levels between 8.5 and 10.5 mg/dl. Hypocalcemic symptoms are uncommon unless the level of S. Calcium is less than 8.0 mg/dl [25, 26].

When S. Calcium levels return to normal within 6 months after surgery, it is transient or temporary hypocalcemia [25, 27]. If the patient presents with hypocalcemia within 24 hours of surgery, it is termed Immediate Hypocalcemia [25, 27]. Delayed hypocalcemia refers to presentation after the first 24 hours post-operatively [25, 27]. Permanent or definitive post-operative hypocalcemia refers to hypocalcemia that persists for 6 months post-operatively or longer [25, 27].

Hypocalcemia commonly presents on the 2nd or 3rd post-operative day. The various clinical features of hypocalcemia depending on the organ system affected are listed overleaf in Box 1 [27].

BOX 1: CLINICAL FEATURES OF ACUTE HYPOCALCEMIA

Neuromuscular irritability:

Symptoms

Paresthesias - acral and facial tingling and numbness
Muscle weakness
Muscle cramps, felt in the limbs predominantly
Tetany
Laryngeal spasm - Stridor
Bronchospasm
Seizures – focal, petit mal or grand mal

Signs

Trousseau's sign: Flexion and adduction of carpal and metacarpo-phalangeal joints induced by supra-systolic occlusion of the brachial artery.
Chvostek's sign : Twitch of the angle of mouth, the nasolabial fold, nasal ala or half of the face including forehead on flicking the angle of the lower jaw

Neurological features:

Symptoms

Personality disturbances, Anxiety and Irritability
Choreo-athetosis and Dystonic spasms
Confusion, Disorientation, Psychosis

Signs

Non-specific EEG changes
Increased intracranial pressure - Papilledema

Cardiac involvement:

Qt prolongation on ECG
Cardiac failure

Smooth muscle involvement:

Dysphagia
Dyspnea
Biliary colic
Abdominal pain

Chvostek's sign and Trousseau's sign deserve a special mention because of the fact that they are the measures most often employed for objective clinical assessment of calcemic status.

Chvostek's sign was first described in 1876 by Frantisek Chvostek, an Austrian surgeon. Elicited by flicking the skin over the angle of the mandible and looking for twitches over the face, it refers to twitching of the muscles of the face as a result of direct mechanical stimulation of the motor fibers in the facial nerve. If twitches are seen, then the sign is positive. This sign has been graded on the basis of how widespread the twitches are over the face.

Chvostek's sign has been graded according to severity [28]. Chvostek I, the severest and most acute degree of positivity, refers to twitches seen over the whole of the side of the face, including the forehead, the nasolabial fold, nasal ala and the angle of the mouth, which can be elicited by light stroking of the finger over the angle of the mouth. Chvostek II refers to twitches seen over the ipsilateral naso-labial fold and the nasal ala. Chvostek III, the mildest degree of positivity, refers to twitch seen over the ipsilateral angle of the mouth alone.

However, the grade of positivity does not correlate with the degree of severity of hypocalcemia. Nor is Chvostek's sign always positive in patients with hypocalcemia. In upto 29% of cases of bio-chemically

confirmed post-operative hypocalcemia, Chvostek's sign is falsely negative. In addition, conditions like rickets, myxedema, alkalosis and some acute infections like diphtheria, pertussis and measles include some of the causes of false positivity of Chvostek's sign. Thus, with a false-positivity of 25% and a similarly high false negativity rate, a positive Chvostek's sign is not a very accurate indicator of hypocalcemia.

Trousseau's sign is elicited by occlusion of the brachial artery with supra-systolic pressure applied by means of the cuff of a sphygmomanometer. If the hand assumes the 'accoucher's' position (flexion and adduction of the carpal, carpo-metacarpal and the metacarpo-phalangeal joints, along with various degrees of flexion of the inter-phalangeal joints) on vaso-occlusion, then the test is positive. However, this is of significance if these changes are seen within two minutes, or 120 seconds, of vaso-occlusion. If the occlusion is maintained longer, the changes are seen even in healthy subjects. A positive Trousseau's response can be graded on the basis of whether changes are seen within 30 seconds, between 30 and 60 seconds or between 60 and 120 seconds after vaso-occlusion.

2.1.4 Management of post-operative hypocalcemia

There are two aspects of management of post-operative hypocalcemia including prevention and treatment of the condition, once it occurs.

Prediction of impending hypocalcemia is vital in prevention of this eventuality. Various studies have been conducted to establish indicators for prediction of post-operative hypocalcemia. If hypocalcemia can be predicted before clinical presentation, prophylaxis can be started so that clinically overt hypocalcemia is forestalled.

Development of hypocalcemia and hypoparathyroidism can be predicted within 24 hours of surgery by estimating S. Calcium and S. PTH levels on the first post-operative day. When calcium levels were plotted as function of time [29], the slope of change of total S. Calcium between 8th & 14th post op hour in hypocalcemic patients was significantly steeper than that in normocalcemics patients. Serial estimations of S. Calcium were performed on an hourly basis. The Calcium slope was calculated as shown below:

$$\text{Calcium slope} = \frac{\text{Calcium 2} - \text{calcium 1}}{\text{Calcium 1 (Time 2 - Time 1)}}$$

Calcium 2 refers to the sample taken later, at Time 2 after operation, and Calcium 1 refers to the sample taken earlier, at Time 1 after operation.

In the post-operative period, calcium levels start to fall after 24 hours of surgery, reaching a nadir 72 hours after surgery, after which the levels rise again. So, estimation of S. Calcium on the first, second and the third post-operative day will enable the detection of biochemical hypocalcemia.

Treatment involves a spectrum of measures ranging from bolus intravenous calcium injection and intravenous infusion through oral calcium and vitamin D supplementation to oral calcium supplementation alone. Grading of the severity of hypocalcemia affords optimization of therapy. Hypocalcemia can be graded on the basis of clinical features, or more exactly, on the basis of S. Calcium.

Severity of hypo-calcemia is graded on the basis of S. Calcium levels in the circulation. Eucalcemia refers to S. Calcium levels between 8.5 mg/dl and 10.0 mg/dl. Mild hypocalcemia refers to S. Calcium levels between 8.0 mg/dl and 8.4 mg/dl. Moderate hypocalcemia refers to S. Calcium levels between 7.5 mg/dl and 7.9 mg/dl. Severe hypocalcemia refers to S. Calcium levels below 7.0 mg/dl. Life-threatening hypocalcemia refers to S. Calcium levels below 7.0 mg/dl.

While oral calcium supplementation is adequate for mild hypocalcemia, moderate or severe hypocalcemia, characterized by S. Calcium levels below 8.0 mg/dl, necessitates intravenous calcium

replacement. No correlation has been established between severity of symptoms and the degree of hypocalcemia.

However, Trousseau's sign correlated with severe hypocalcemia. Those patients with positive Trousseau's sign require intravenous replacement, in the form of a bolus followed by intravenous infusions. Continuous monitoring of pulse and ECG and periodic monitoring of S. Calcium levels is essential during intravenous calcium replacement. Once S. Calcium is consistently more than 8.0 mg/dl, the infusion can be tapered and stopped. Oral supplementation has to be continued for a period of 4-6 weeks.

2.1.5 Permanent hypoparathyroidism and chronic hypocalcemia

While hypoparathyroidism and the resultant hypocalcemia is eminently treatable, permanent hypoparathyroidism is one of the most crippling of complications after thyroid surgery. Symptoms and signs of hypocalcemia apart, chronic hypocalcemia places the patient at risk of significant long-term morbidity, including metastatic calcification of the basal ganglia causing seizures and extrapyramidal symptoms [30], calcification of the lens capsule, resulting in cataracts [31], reversible peripheral neuropathy [32] and hypocalcemic heart failure [33]. The clinical features of chronic hypocalcemia are listed in Box 2 [27, 34].

Considering all the above-said factors that influence post-operative hypoparathyroidism, the best approach to prevention, especially for surgeons, is by means of identification and preservation of the parathyroid glands and their blood supply or by autotransplantation of one of the parathyroid glands.

BOX 2: CLINICAL FEATURES OF CHRONIC HYPOCALCEMIA

Neurological signs and symptoms

Extrapyramidal signs due to calcification of basal ganglia

Calcification of cerebral cortex or cerebellum

Parkinsonism

Ectodermal changes

Skin: Dry skin, Coarse hair, Brittle nails, Alopecia, Atopic eczema, Exfoliative dermatitis, Psoriasis, Impetigo herpetiformis

Teeth: Enamel hypoplasia, Shortened premolar roots, Thickened lamina dura, Delayed tooth eruption, Increased dental caries

Ophthalmologic manifestations

Subcapsular cataracts

Cardiac

Congestive heart failure, Cardiomyopathy

2.2 PREVENTION OF POST-OPERATIVE HYPOPARATHYROIDISM:

PRESERVATION OF PARATHYROIDS ON THE OPERATING TABLE

Accurate recognition and preservation of normal, vascularised parathyroids is dependent on various factors, the foremost of which are surgical experience, subjective visual assessment, and a thorough knowledge of the gross anatomy of parathyroid glands including blood supply.

2.2.1 Anatomy and embryology of the parathyroid glands

It is common knowledge that there are four parathyroid glands, in the neck, with a superior and an inferior gland on each side of the midline. Super-numerary parathyroid glands are seen in upto 13% - 15% of the subjects, while 1-3% of the subjects have been reported to have fewer than 4 glands [35].

These parathyroid glands are embryologically derived from the pharyngeal pouches – the inferior glands from the third pharyngeal pouch and the superior parathyroids from the fourth pharyngeal pouch.

As the thymus descends into the lower neck and thorax, the parathyroid glands are dragged down and so, come to assume a caudal position with respect to those that develop from the fourth pouch. The

superior parathyroid glands derived from the fourth pharyngeal pouch, develop in the neck. The postero-lateral aspect of the thyroid lobe develops from the ventral bud of the fourth pharyngeal pouch on each side and thus, the superior parathyroids develop in close proximity to the lateral thyroid anlage.

As a result, the superior parathyroids assume a definitive position in close proximity to the postero-lateral border of the thyroid lobe. Invariably, the superior glands are enclosed within a thin fascial sheath, a so-called capsule, which is continuous with the thyroid capsule. This sheath is sometimes vascular. The position of the superior glands is relatively constant.

In a landmark study on parathyroid anatomy based on cadaveric dissection [35], 80% of the superior parathyroid glands were found near the junction of the cricoids and thyroid cartilages, within 1 cm from the intersection of the recurrent laryngeal nerve and the inferior thyroid artery.

They are usually found posterior to the upper pole of the thyroid lobe. Sometimes, the glands can be more anterior in position, on the surface of the gland within the thyroid capsule.

The inferior parathyroid glands have a longer path of descent when compared to the superior glands and so, it follows that the inferior glands

are more variable in position. They are not as closely related to the thyroid lobe as the superior glands. They are more variable in their distribution. Akerstrom, et al. [35] found the glands in close relation to the inferior pole of the thyroid lobe in 44% of subjects. In a further 28%, the glands were found in relation to the cervical pole of the thymus or within the thyro-thymic ligament in the lower neck. Anomalous development of the third pouch can result in an abnormally high location of the gland, near the carotid bifurcation.

A diagrammatic representation of the anatomical variations and the frequency with which these variations occur in the population is depicted in Figures 1 and 2. The superior and the inferior parathyroid glands and their relation to the RLN and ITA are as shown in Figure 3.

The thin fascial sheath overlying the superior as well as the inferior parathyroid glands, which is invariably seen, renders them mobile within their capsule, unlike nodules of the thyroid itself or perithyroidal fat or lymph nodes. This freedom of movement is one of the key points for identification of parathyroids on the operating table.

Intra-thyroidal parathyroids have been described [35]. The fourth pharyngeal pouch, which gives rise to the superior parathyroid gland, also gives off the lateral thyroid complex, which gives rise to the lateral part of the thyroid lobe. So it should follow that the superior parathyroid

glands are more likely to be found in an intrathyroidal location, as was postulated [36].

However, another group of researchers [37] reported on a subset of intrathyroidal parathyroid tumours among a series of patients who were operated. 7 of the 8 tumours reported were considered to be arising from the inferior parathyroid glands.

Overall, the incidence of intrathyroidal parathyroid glands has been reported to be ranging between 0.5% and 3% [35, 36, 38, 39].

2.2.2. Size, shape and colour of the viable, eutopic parathyroids

Proper identification requires definition of shape, size and colour of the glands as well, in addition to the anatomical position. Because a significant part of the gland and the cells are made up of lipids, which are fluid at body temperature, these glands are soft in consistency and can be easily moulded. The shape is dependent upon the anatomical location and the state of the adjacent structures. Those glands that are located in loose areolar tissue have an oval, tear-drop or a bean-shaped configuration. Those that lie in close relation to the thyroid capsule, especially if the thyroid is enlarged, appear flattened with sharp edges. Sometimes, the glands can appear elongated. Generally, hyper-functioning glands appear rounded and spherical.

Although there is a variation in the size of the normal parathyroid gland, glands which are upto 6-8mm in the longest dimension are physiologically normal.

The colour of normal parathyroid glands ranges from yellowish brown to reddish brown. Sometimes, they appear pinkish in contrast to the surrounding peri-thyroidal fat. The amount of intra-glandular fat, the number of oxyphil cells and extent of vascularity are factors which influence the colour of the parathyroid glands [40].

2.2.3. Blood supply of parathyroid glands

Due to their rich vascularity and fine, delicate vessels, inadvertent handling of the glands sometimes leads to bruising of the glands, which is apparent on the table as a dusky blush within the parathyroid capsule.

Meticulous preservation of the anatomy alone without attention to preservation of vascularity will anyway result in loss of functionality. While it is traditionally believed that most of the glands are supplied by a single end-artery, there is a single arterial supply in 80% of parathyroid glands, a double arterial supply in 15%, and multiple arteries in 5% [41].

In general, the parathyroid glands, regardless of whether superior or inferior, derive blood supply from the inferior thyroid artery. However, the superior thyroid artery perfuses upto 20% of the superior parathyroids

and 10% of the inferior parathyroids. Whatever be the origin of the parathyroid arteries, these run in the perithyroidal tissue before entering the gland. While ligating the thyroid pedicles, it is preferable to avoid ligation of the main trunks of the arteries. A third source of blood to these glands is via capsular vessels that run between the thyroid and parathyroid glands. This is evident during thyroidectomy, when dissection in the interglandular plane may result in bleeding.

In the event of unavoidable or inadvertent parathyroidectomy, the gland which has been separated is autotransplanted into skeletal muscle. This is possible due to certain properties of the parathyroid gland that render this a viable option.

2.2.4. Floatation test

In case of inadvertent parathyroidectomy, it is often not possible to differentiate the separated parathyroid gland from peri-thyroidal fat. This can be facilitated by immersion of the tissue in question in saline. Fat, with a relative density less than that of water, will float to the surface. The gland, which has a relative density higher than that of water, will sink to the bottom. This is a crude indicator of whether the tissue in question is a parathyroid gland or not. The definitive test of confirmation

would be a histopathological examination, or at least, microscopic examination of a frozen section of the tissue.

Thus, the various anatomical attributes by which parathyroid glands can be identified on the operation table include the position with relation to the thyroid gland and its capsule, relation to the RLN and the ITA, size, shape and colour of the glands, Intracapsular mobility, bruisability and blood supply of the parathyroid glands. Flotation test will enable identification if inadvertent parathyroidectomy occurs. However, the definitive method of identification is microscopic examination of a frozen section sample of the harvested tissue.

2.3 PARATHYROID PHYSIOLOGY, PHARMACOKINETICS AND PTH ASSAYS

Parathormone is a polypeptide, made up of 84 amino-acid residues, secreted by the parathyroid glands. It is synthesized primarily in the chief cells as pre-pro-parathormone, a precursor with 115 amino acid residues. As a matter of convention, the amino-acid residues in peptides and proteins are numbered from the amino-terminal towards the carboxy-terminal. The native hormone is then metabolized in the liver to amino-terminal and carboxy-terminal fragments. The amino-terminal fragment, involving the first 27 to 34 amino acid residues, is the biologically active part of PTH. The other fragments of PTH are generally inactive biologically. While assaying the activity of PTH, measurement of biologically active PTH species holds more relevance than measurement of inert fragments of the hormone.

While this is the logical approach to assessment of PTH function in theory, it is not so in practice, due to certain pharmaco-dynamic processes in the metabolism of PTH. The pre-pro-hormone, which has 113 amino-acid residues, is the product of the human PTH gene, undergoes changes within the chief cell, ultimately resulting in cleavage of the 29-residue moiety, resulting in the mature, 84-residue PTH molecule. This is then concentrated into secretory vesicles and stored for secretion when

necessitated. In addition, other intracellular forms of PTH, like some metabolites like amino- and carboxy-terminal peptides, are secreted into the circulation.

This process of intracellular metabolism of PTH is regulated by the ambient calcium level in blood. When the level of ambient calcium is low, the predominant form secreted is the mature, native molecule (PTH 1-84). When secretion is suppressed by high calcium levels, most of the secretion is in the form of fragments. Once in circulation, these molecules are metabolized in the liver and other peripheral sites like the kidneys and the bones into peptide fragments, which are found in the circulation [44]. So it follows that there are several species of PTH in the circulation including the intact PTH, amino-terminal (N-terminal), carboxy-terminal (C-terminal) and mid-region fragments [42, 43, 45].

Each of these types of hormone is detectable by immunoassay procedures. The intact hormone makes up about 10% - 20% of the circulating immuno-reactive PTH. The remainder is a mixture of other peptide fragments of the PTH molecule.

These circulating forms of PTH are ultimately cleared from the circulation by glomerular filtration [42]. So, renal function has a significant influence on the level of circulating PTH forms and their assays in the blood.

The intact hormone and its N-terminal fragments disappear rapidly from the circulation, with half-lives lasting minutes, while the C-terminal and mid-region fragments, although biologically inactive, have longer half-lives of hours [43, 46].

Peptides, by nature are heat-labile. So it was traditionally believed that estimation of PTH, a labile hormone, necessitated freezing of serum as early as possible, in order to obtain reliable results. However, it has been found that upto 90% of the immuno-reactivity of PTH is retained for upto 4 hours at room temperature, unless the patient has pancreatitis, which increases protease activity.

So it should follow that a delay in the separation of serum and freezing doesn't have a major effect on assay. This is true to an extent. Nevertheless, blood samples should be processed within a reasonably short time to minimize hormone degradation. Some assay procedures employ the usage of enzyme inhibitors in collecting tubes, thus prolonging the shelf-life of collected blood.

There are two general formats for these assays – the older, one epitope-site solution assays and the newer, two epitope-site solid phase assays.

In the solution assay, antiserum containing a known anti-PTH antibody is taken, to which two types of sera are added, the one with

known amount of radiolabelled PTH and the other with an unknown amount of the patient's PTH. It is based on competition between the known, radiolabelled PTH and the unknown test PTH for the antibody sites.

The two-site assay or the sandwich assay employs two antibodies with separate recognition sites on the antigen. These assays are also referred to as immunometric assays. The principle had been recognised for years, but advent of enhanced production of monoclonal antibodies has enhanced the development of these assays and their clinical application. In this assay, one of the antibodies is usually attached to a solid matrix like beads or micro-titre wells, while the other antibody is radiolabelled. The PTH antigen is the filling in this sandwich.

The two-site assay has many advantages, including the ability to directly measure specific and defined forms of PTH, increased sensitivity even with low-affinity antibodies and absence of non-specific protein artefacts, which decrease the accuracy of assays using blood [47-49].

Intact PTH assays (iPTH) are sandwich assays in which one of the antibodies is directed against the N-terminal, while the other is directed against the C-terminal. A synthetic peptide version is used as the PTH standard. With these assays, it is possible to estimate the amount of mature, 1-84 PTH.

2. 4. PARATHYROID AUTO-TRANSPLANTATION

Intact, viable parathyroids are indispensable for normal calcium homeostasis, but as is evident, hypoparathyroidism does occur as a complication of thyroid surgery. However, the parathyroid glands have certain properties, like the ability to survive by imbibition initially and later form blood vessels of their own. Thus, they survive, grow and function after transplantation, preventing definitive hypoparathyroidism and chronic hypocalcemia.

2.4.1. History of parathyroid auto-transplantation

Parathyroid Auto-transplantation (PTAT) has been reported for over a century. While it was reported in animal studies in 1907, the first reported parathyroid autotransplant in a human being was performed by William Halsted in 1909, to avoid hypoparathyroidism.

PTAT during thyroid surgery was first reported in 1926, when it was performed during partial thyroidectomy by Lahey [50]. The first experience of parathyroid autotransplantation after total parathyroidectomy was described in 1968 [51].

Viability of the auto-transplanted parathyroid was first reported in 1975. Wells, et al. [52] reported clinical function and normal histology of

the grafts in a large series of patients undergoing PTAT during thyroid surgery. In the same year, Hickey and Samaan [53] showed that autotransplanted parathyroid glands secrete PTH.

With time, people started acquainting themselves with the techniques of parathyroid autotransplantation and cryopreservation. Gradually, these methods gained popularity as measures to lower rates of hypocalcemia and hypoparathyroidism following total thyroidectomy.

Then, as details of parathyroid blood supply were better understood, measures like avoidance of truncal ligation of the thyroid arteries were described and advocated. As this procedure gained acceptance, the rates of identification and preservation of the parathyroid glands increased. As a result, the rates of parathyroid gland injury and necrosis decreased drastically. While this led to a marked reduction in the rates of permanent hypoparathyroidism complicating thyroidectomy, the incidence of transient hypocalcemia has remained high.

2.4.2 Imbibition

Immediately after PTAT, the autotransplanted tissue survives by means of imbibition. This refers to the process of passive diffusion of water, oxygen and nutrients into the cells of the graft from the surrounding tissue fluid. The metabolic wastes diffuse out into the

surrounding fluid. Grafts can survive by imbibition for upto a week after transplant. The key factors which influence survival of the graft by imbibition are the degree of perfusion of the graft bed and the size of the bits of tissue that are implanted. There are many reports [56-59] of successful PTAT after transplantation of the tissue within the belly of skeletal muscle, a tissue which is well-perfused and well-oxygenated.

The bits of tissue that are grafted should be small enough to survive the immediate post-operative period, before neo-vascularization. Successful PTAT has been reported with various sizes of the grafts [60-62].

Transplantation of bits of tissue with a volume of 1mm^3 ($1\times 1\times 1\text{mm}$) ensures optimal survival of the tissue. With time, new blood vessels start to develop within the graft by virtue of angiogenesis. Various factors influence this process.

2.4.3 Angiogenesis

It goes without saying that viability and functionality of a transplanted organ or bit of tissue is dependent on reestablishment of a functional blood supply. In case of transplant of whole organs like the kidney or large volume of tissue, like the liver, graft viability and function is based on establishment of intact vascular anastomoses.

However, in case of parathyroid autotransplantation, procedures like vascular or micro-vascular anastomosis are not performed. This is mainly because it is not required, thanks to certain properties exclusive to the tissue. The ability of the parathyroid tissue to induce angiogenesis has been demonstrated in vitro [63] as well as in vivo [64].

Vascular endothelial growth factor (VEGF) has been shown to contribute to the development of the angiogenic phenotype in vitro [65]. Angiogenic activity was observed during the first post-operative week in athymic mice which were subjected to parathyroid autotransplantation [66]. In addition, reinnervation along newly built blood vessels has been demonstrated in transplanted parathyroid tissue 1 week post-operatively [67].

In a trans-species study [60], parathyroid tissue that was harvested from patients was cut into 2x2x1 mm pieces, which were subsequently transplanted into nude mice. Angiogenesis induced by these grafts was detectable by light microscopy on the 5th day after the procedure. Newly grown microvessels were seen to be originating from host venules. Human iPTH was detected in plasma samples of the mice. So it follows that the donor microvessels served as pathways for sprouting microvessels.

Apparently, vascular ingrowths develop in about 10-20 days following implantation [68]. Graft function mirrors this process as well and is reflected in serum PTH levels taken during this period.

So various researchers have reported evidence of angiogenesis and neovascularization in the parathyroid implants at various times after PTAT, as summarized below.

Researcher	Time after PTAT for angiogenesis
Ander SJ, et al.	During the first post-operative week
Luts and Sundler	1 week post-operatively.
Strieth, et al.	5 th day after the procedure
Wells SA Jr, et al.	10-20 days following implantation

2.4.4 Site for transplantation

A well-perfused, vascular bed, like skeletal muscle belly, is necessary for autotransplantation in order for the graft to survive by imbibition before new blood vessels are formed by angiogenesis. Generally, the preferred sites for transplantation are the musculature of the forearm and the sternocleidomastoid muscle [59, 69-71].

According to Walker, et al. [72], the need to re-enter the neck to excise hyperfunctioning parathyroid tissue is extremely uncommon in

case of PTAT during thyroidectomy, when the parathyroids are not diseased. So, the advantage of employing the sternocleidomastoid is that another incision can be avoided. However, there are various reports of PTAT into the belly of the brachioradialis muscle in the forearm [61, 62, 73]. It is much easier to assess graft function.

Funahashi, et al. [59] recommend transplantation of the parathyroids into the pectoralis major when a lymph node dissection is required for malignancy. The advantage of this method is that the pectoralis major is directly accessible through the neck incision. During neck dissection, when the sternocleidomastoid muscle is taped and freed completely, the blood supply to the muscle may be jeopardized, at least transiently. This may adversely affect graft viability.

In a study by Lo, et al., the harvested parathyroid gland was minced into fragments of 0.5 mm size [61]. These were inserted into multiple pockets in the brachioradialis muscle, following which survival and functionality of the grafts was documented.

There is a report of successful pre-sternal subcutaneous autotransplantation of parathyroid glands. In their study, Kinnaert, et al. [74] found that the late results, with respect to graft viability, of total parathyroidectomy with pre-sternal subcutaneous autotransplantation

compared favourably with published data on other forms of surgical treatment of renal hyperparathyroidism.

So, while there are advocates of various sites within the body for implantation of harvested parathyroids, they all concur on the fact that belly of the skeletal muscle is a suitable site for PTAT.

2.4.5 Mode of transplantation

The key to successful graft survival during the first few days after PTAT is imbibition. It follows that the smaller the graft, the more the chances of survival by imbibition. Billings and Milroy [73] have reported successful transplants in which the harvested parathyroid tissue was minced in iced Waymouth's tissue culture medium. The resulting suspension was transplanted into the deltoid and brachioradialis muscles, by means of placement into pockets as well as by injection into the muscles.

In another study [62] in patients with chronic renal disease and parathyroid hyperplasia, total parathyroidectomy and PTAT was performed successfully and graft viability was reported. The gland which was to be auto-transplanted was sliced into 30 pieces, each of size 1x1x3 mm. These bits were implanted into 30 pockets in the brachioradialis muscle of the forearm.

Testini, et al. [24], in their study on the impact of PTAT during thyroidectomy, employed a technique in which the harvested parathyroid glands were sliced into 1x2mm pieces. One of them was sent for histological confirmation, while the rest of them were immediately autotransplanted into an intramuscular pocket in the sternocleidomastoid on the side of harvest. The site of transplantation was closed with a non-absorbable suture to prevent extrusion of the graft. The grafts were reported to be functional and viable.

Wells SA Jr., et al. [75], in their report about long-term follow-up after PTAT during thyroidectomy, reported that the harvested parathyroid gland was sliced into pieces of size 1x3mm, which were implanted into muscle pockets in the sternocleidomastoid muscle. These were found to be functioning in the long term.

Delbridge, et al. [76] conducted a prospective randomized controlled trial, to compare the outcomes with respect to the procedure of parathyroid autotransplantation. The conventional method of implanting bits of parathyroid tissue was compared with a novel method of injecting a suspension of finely minced parathyroid tissue into the muscle bulk (Milroy technique). Post-operative assessment included clinical assessment together with estimation of S. Calcium and intact parathormone (iPTH) immediately before the procedure and again on

post-op days 1, 14 and 90. In both the groups, the procedure was followed by a fall in mean PTH levels, but it was significant in the implantation group. By 2 weeks, the calcium and iPTH levels had returned to baseline levels. None of the patients developed permanent hypoparathyroidism.

2.4.6. Number of parathyroids to be grafted

Another consideration is the number of parathyroids that can or should be autotransplanted during thyroidectomy. It is a matter of general consensus that up to two glands can be removed without significantly increasing the risk of post-operative transient hypoparathyroidism, discounting other factors that influence this outcome.

A couple of studies have compared parathyroid preservation with routine autotransplantation of all visualized glands [77, 78]. In the subset of patients who underwent routine autotransplantation, incidence rates of permanent hypoparathyroidism were 43% and 21.4%.

According to Imai, et al. [79], the key to avoid post-op hypoparathyroidism after total thyroidectomy with PTAT in patients with papillary thyroid carcinoma is to find and transplant more than 3 PTGs.

Various researchers [4, 80] have demonstrated that the number of parathyroids transplanted correlated with incidence of transient

hypoparathyroidism and post-operative hypocalcemia. However, there was no correlation between the number of glands transplanted and permanent hypoparathyroidism [80].

The removal of a well-vascularized and otherwise normal parathyroid gland is unnecessary. It does not reduce the incidence of permanent hypoparathyroidism [4].

So it follows that autotransplantation should only be performed in cases of inadvertent parathyroidectomy or devascularisation of the glands. Even if performed prophylactically, there is definitely no case for autotransplantation of all visualized parathyroids.

2.4.7. Preservation of harvested tissue during surgery

In their study, Wells SA, Jr. [75], et al. took care to auto-transplant the bits of parathyroid tissue into the graft beds within 120 minutes of harvesting, to ensure that the grafts were viable. The parathyroid tissue was put into iced saline immediately after harvesting.

Another effect of preservation of tissue in iced saline is that the tissue firms up, probably due to solidification of the fat within the tissue. This makes it easier to slice or mince the tissue.

So it is advisable to put the gland to be transplanted in iced saline immediately after harvesting, until the time of auto-transplantation, which is best performed within 120 minutes of harvesting.

2.4.8. Methods of assessment of graft function

The overwhelming majority of patients undergoing PTAT have hyper-parathyroidism. The surgery involves removal of all of the functioning parathyroid tissue from the body followed by PTAT, invariably in the sternocleidomastoid muscle. Objective determination of graft function by means of PTH assays is possible since the graft is the only functioning parathyroid tissue in the body.

On the other hand, in the case of total thyroidectomy, functioning parathyroid tissue is left in the neck. Traditionally, determination of graft function has been based on the resolution of clinically significant hypocalcemia or on the recovery pattern of parathyroid hormone (PTH) level [61].

Most authors [74, 81, 82] favour the forearm as the site for transplanting parathyroid tissue. Estimation of S. PTH from both the antecubital fossae is easily done. Graft function can be easily assessed by evaluating the PTH gradient between the two sides.

Lo CY, et al. [61] have documented graft function in PTAT following thyroidectomy. The grafts were implanted in to the

brachioradialis. S. PTH was estimated in samples from both the antecubital fossae. By the 7th week after PTAT, a significant gradient between the test and the control arms was established. It seemed that normal parathyroid tissue resumed function 2 to 4 weeks after re-implantation.

Significant iPTH gradients can be demonstrated in about 80% of the patients after autotransplantation [83]. Pre-dilution of the samples to 1:10 and 1:100 enabled more accurate measurements of iPTH levels [84].

Another method of assessment of graft function, the modified Casanova test, was described in the setting of recurrent hyperparathyroidism in cases of forearm autotransplantation [85]. However, this is not applicable to patients undergoing thyroidectomy with normal parathyroid function.

2.4.9. Time of assessment of function

Various researchers have studied this aspect of PTAT. According to Takagi, et al. [86], the gradient of PTH is usually significant (>1.5) within 2 or 3 weeks postoperatively.

Freidman, et al. [15] reported full recovery of parathyroid function by the end of 4th week after autotransplantation during thyroidectomy, at

which point a two-fold increase in mean PTH levels was observed. After a further 4 weeks, mean PTH levels had increased by 3-fold.

According to reports from Korea and Turkey [61, 87], the transplanted glands achieve normal function only after 3 to 14 weeks. In another study from Greece [62], the transplanted parathyroid tissue started functioning after 5.2 months on average (range, 1.7-6.1 months).

2.4.10. Test : Control Ratio

In humans, a gradient of 1.5 or greater in PTH measurement between grafted (test) and non-grafted (control) arms has been generally accepted as proof of graft function [64, 75].

In their study of long-term parathyroid function following total parathyroidectomy with PTAT, Imai, et al. [88], found that the average value of iPTH was 36.1 pg/ml in the systemic circulation and 908 pg/ml near the transplantation site, giving a ratio of 25.2.

In the setting of total parathyroidectomy with PTAT, a test-to-control PTH ratio of 2.0 was found to be helpful in assessing viability and function of parathyroid tissue in the forearm [89].

There have been reports doubting the usefulness of test-to-control arm ratio with respect to serum PTH [90]. A hormone gradient between the test and control arms may be disguised by high levels of

parathormone in the systemic circulation [91]. However, in eucalcemic and euparathyroid patients, this ratio is more reliable since the systemic levels of PTH are within the normal range [92].

Consecutive patients who underwent total thyroidectomy in the Department of Endocrine Surgery, Madras Medical College were included in the study.

Pre-operative tests and preparation:

The thyroid status, parathyroid status and renal function were assessed pre-operatively. Those who had hyperthyroidism or hypothyroidism were adequately treated with pharmacotherapy (anti-thyroid drugs or replacement with levo-thyroxine) for a minimum period of six weeks. Decision to proceed with surgery was made after confirmation of clinical and biochemical euthyroidism.

Exclusion criteria:

Patients with renal insufficiency and hyperparathyroidism were excluded. If the whole of the thyroid gland was not removed (hemithyroidectomy), or if the thyroid gland was not eutopic (ectopic thyroid, thyroid hemi-agenesis) such patients were excluded from this study.

Operative procedure:

Informed consent was obtained after the requisite pre-operative preparation and the patients were then posted for surgery. The operative

procedure was standardized and was followed by all the surgeons who operated on the patients.

A low collar crease incision was employed and sub-platysmal flaps were raised. The sternocleidomastoid muscles on either side were reflected to expose the carotid sheath. Neck dissections, if deemed necessary, were performed after exposure of the carotid sheath and deep cervical lymph nodes. The investing layer of the deep cervical fascia was divided in the midline. The sterno-hyoid muscles were retracted laterally and the sterno-thyroid muscles were divided near the cranial attachment. The number, position, colour, shape and vascularity of parathyroids were recorded. If three or more parathyroid glands with normal morphology and intact blood supply were seen, one of them was harvested and placed in 2 ml of ice-cold saline. The thyroid was removed after identification and preservation of the RLN and the remaining parathyroids.

Graft processing:

A small part of the harvested parathyroid gland was set aside and sent for histological confirmation. The remaining gland was finely minced to particles of size 1mm^3 or smaller, as shown in Figure (4).

Graft bed preparation and graft implantation:

The skin overlying the belly of the brachioradialis, in the proximal part of the ventero-lateral aspect of the forearm was incised. The brachioradialis was exposed (Figure 5). Excessive bleeding, if any, was controlled by digital pressure. Care was taken to avoid diathermy over the muscle belly. The parathyroid-saline suspension was then injected into the graft bed through a wide bore (14 guage) needle (Figure 6). The wound was closed with non-absorbable sutures after ensuring that there was no extrusion of the graft suspension.

Care was taken to perform the autotransplantation within 120 minutes of harvesting the parathyroid gland.

Post-operative monitoring:

Features of RLN palsy (aspiration, change of voice, hoarseness, vocal cord palsy on video-laryngoscopy), if present were noted. If and when clinically overt hypocalcemia presented, (muscle weakness, perioral and acral paresthesias, Chvostek's sign, Trousseau's sign, post-op S. Calcium) the above-said findings were noted and S. Calcium estimated. In asymptomatic patients, the S. Calcium was estimated on the third post-operative day. Biochemical hypocalcemia ($S. Ca < 8.5 \text{ mg/dl}$), if present, was recorded.

Severity of hypocalcemia was graded according to the clinical features and post-operative level of S. Calcium as given below.

Grading of severity of hypocalcemia:

Chvostek's sign:

Chvostek's I – Twitches over the angle of the mouth, the nasal ala, the nasolabial fold and the forehead

Chvostek's II – Twitches over the angle of the mouth, the nasal ala and the nasolabial fold

Chvostek's III – Twitches over the angle of the mouth alone.

Trousseau's sign:

Trousseau's I – Spasm between 1-2 minutes after vaso-occlusion

Trousseau's II – Spasm between 30-60 secs after vaso-occlusion

Trousseau's III – Spasm within 30 secs of vaso-occlusion

S. Calcium:

S. Ca 8.5 mg/dl or above – Eucalcemia

S. Ca b/w 8.0 and 8.4 mg/dl – Mild hypocalcemia

S. Ca b/w 7.5 and 7.9 mg/dl – Moderate hypocalcemia

S. Ca 7.5 mg/dl or below – Severe hypocalcemia

S. Ca 6.0 mg/dl or below – Life-threatening hypocalcemia

Management of hypocalcemia:

Patients with moderate or severe hypocalcemia post-operative hypocalcemia and those with positive Trousseau's sign were treated with intravenous infusion of 10% Calcium gluconate. Simultaneous oral supplementation with calcium and 1,25-dihydroxycholecalciferol (1,25-DHCC) was started. Clinical features including symptoms of hypocalcemia, Trousseau's sign and Chvostek's sign were monitored once in every 6 hrs. Infusion was tapered and terminated after confirmation of stabilization of S. Calcium (8.0 mg/dl or more) and stabilization of Trousseau's sign (two consecutive negative responses). Those patients with mild hypocalcemia were treated with oral supplements as above. The patients were discharged when deemed fit.

Assessment of graft function:

After a minimum of 90 days following surgery, the patients were reviewed. Simultaneous bilateral cubital vein sampling was done and S. PTH was estimated in the samples. The graft was deemed viable if the test-to-control S. PTH ratio was at least 1.5. All those patients in whom the ratio was less than 1.5 were reviewed 6 months after the procedure. Blood samples from the test and control arms were taken again and levels of PTH were estimated. If the test-to-control ratio was less than 1.5 after

6 months, the graft was deemed nonfunctional. Findings were noted, tabulated and analyzed.

Statistical Analysis

After tabulating, the data were analyzed with regards to graft function after six months. Graft function was correlated with thyroid pathology, presence of thyroiditis, location of the harvested gland, type of procedure performed and the cold-ischemia time of the graft.

Analysis was performed using the Chi-squared test with Pearson's correlation. Multivariate analysis was performed with the help of logistic regression.

Statistical analysis was conducted with the help of the SPSS software (version 17.0).

This study was conducted over a period of 18 months from June 2011 to December 2012.

Out of the 57 patients who were initially included in the study, 4 parathyroids were identified in 45 patients, while 3 parathyroids were identified in a further 6. In the remaining 6 patients, only two glands were identified and so, harvesting and autotransplantation was abandoned. Out of the 51 patients in whom parathyroid autotransplantation was performed, three were lost to follow-up. A total of 48 patients who were followed-up with post-operative PTH estimation were included in the final analysis.

Demographics

The mean age of the patients included in this study was 41.1 yrs \pm 13.7. The youngest patient was 17 yrs old, while the oldest patient included in this study was 75 yrs old. Of the 48 patients who were included in this study, 42 (87.5%) were women and 6 (12.5%) were men (Figure 4).

Thyroid status

As regards to thyroid status, the majority of patients (26 of 48, 54.2%) were euthyroid, while 9 of them (18.7%) were hyperthyroid at

diagnosis and the remaining 13 (27.1%) were hypothyroid at diagnosis. Of the 9 patients who were hyperthyroid, 7 of them had benign thyroid disease and 2 of them had papillary thyroid carcinoma.

Pathology of the thyroid disease

With respect to thyroid pathology, the majority of them (28 out of 48, 58.3 %) had non-toxic nodular colloid goitre. A further 7 of them (14.6%) had diffuse goitre. 6 patients (12.5%) had toxic multinodular goitre and the other 7 patients (14.6%) had thyroid malignancy [6 patients with papillary thyroid carcinoma (PTC) and one patient with medullary thyroid carcinoma]. As already mentioned, two of the patients with PTC were hyperthyroid at the time of diagnosis.

In 17 out of 48 patients (35.4%), chronic thyroiditis was evident in the final histology, either as the predominant feature or as a co-existing feature with the dominant histology.

Type of procedure performed

Total thyroidectomy (TT) was performed in 41 patients (85.4%). Five patients (10.4%) underwent total thyroidectomy with central lymph node dissection (CLND). MRND was performed in addition to TT and CCLND in two patients (4.2%).

Location of the harvested gland and graft bed

The harvesting of the graft was heterogeneous, with right inferior parathyroid gland harvested in 33.3% (n=16), followed by the left inferior gland in 27.1%(n=13), the left superior parathyroid in 25%(n=12) and the right superior parathyroid in 14.6%(n=7) of the patients.

The graft was implanted in the right forearm in 23 out of the 48 patients (47.9%) and in the left forearm in the rest of the 25 patients (52.1%).

Functionality of graft

Overall, of the 48 patients who were included in this study, the autotransplanted parathyroid was found functioning, as evidenced by a test-to-control ratio of 1.5 or above, in 35 patients (72.9%). In the remaining 13 patients (28.1%), the graft was deemed non-functional after 6 months of autotransplantation. The PTH values and the test-to-control ratios were as shown in Figures 7 and 8.

Among the 35 patients who had functioning grafts after 180 days, the function was established after 90 days in 29 of them. In the remaining 6 patients, a test-to-control ratio of 1.5 or more was seen by the 180th day after autotransplantation.

When graft functionality and viability was correlated with pathological diagnosis, presence of thyroiditis, site of the gland harvested, procedure performed and cold ischemia time, the findings were as described under.

With respect to pathological diagnosis (Table 1), the rate of graft viability and functionality was 75% in patients with non-toxic, nodular colloid goitre. In patients with diffuse goitre, toxic multinodular goitre and malignancy, it was 28.6%, 83.3% and 100% respectively. This difference was found to be statistically significant ($p = 0.04$) on univariate analysis. However, on multivariate analysis, this difference was not found to be significant ($p = 0.11$).

Table 1: Correlation of graft viability and function with histopathological diagnosis including thyroiditis

Pathology	Functional grafts, n (%)	Nonfunctional grafts, n (%)	Total
Nontoxic nodular goitre	21 (75.0)	7 (25.0)	28
Diffuse goitre	2 (28.6)	5 (71.2)	7
Toxic MNG	5 (83.3)	1 (16.7)	6
Thyroiditis present	12 (70.6)	5 (29.4)	17
Thyroiditis absent	23 (74.2)	8 (25.8)	31

When correlated with thyroiditis (Table 2), the rate of graft viability and function at 6 months after autotransplantation was 70.6% when thyroiditis was present, as opposed to 74.2% in cases without thyroiditis. Although thyroiditis appeared to be inversely related to graft viability, this observation was not statistically significant ($p = 0.79$).

Of the 29 patients in whom the inferior glands were harvested and autotransplanted, they were functioning after 6 months in 19 of them (65.5%). In the remaining 19 patients in whom the superior parathyroids were harvested, the rate of functionality after 6 months was 84.2 %. However, this difference was not statistically significant ($p = 0.18$, Table 2).

Table 2: Correlation of graft viability and site of harvested gland

Gland harvested	Functional grafts, n (%)	Nonfunctional grafts, n (%)	Total
Left Superior	9 (75.0)	3 (25.0)	12
Right Superior	7 (100.0)	0 (0.0)	19
Left Inferior	10 (76.9)	3(23.1)	13
Right Inferior	9(56.3)	7(43.7)	16

The rate of graft viability was 69% after total thyroidectomy (Figure 8). In patients who underwent total thyroidectomy with lymph

node dissection (CLND, MRND or both), the rate of viability was 100%. Again, the apparent difference was not statistically significant ($p = 0.62$)

The mean time between harvesting and transplantation of the gland was 45 ± 6.5 minutes in those who had functioning grafts after 6 months (Figure 9). That in the subset of patients with nonviable grafts after 6 months was 58.5 ± 13.2 minutes (Figure 10). This difference was statistically significant ($p < 0.001$).

On multivariate analysis, graft survival correlated with cold ischemia time of the harvested gland. None of the other factors considered had any significant association with graft survival rate.

Post-operative sequelae and complications

Hypocalcemia was seen in 18 out of the total number of 48 patients (37.5%). Among them, 8 patients (45.4%) had overt hypocalcemia as evidenced by positive symptoms and signs, while the other 10 patients (55.4%) had clinically occult hypocalcemia.

Clinical symptoms of hypocalcemia, including paresthesias and cramps were seen in 8 patients (Table 2). The commonest symptom was muscle cramp. 6 patients had complaints of paresthesias post-operatively.

Signs of hypocalcemia were evident in all the 8 patients, as described in Table 3.

Table 3: Post-operative hypocalcemia in the present study

Patient	Acral paresthesias	Perioral paresthesias	Cramps	Chvostek's (Grade)	Trousseau's (Grade)
1	+	-	+	2	1
2	-	-	+	1	2
3	+	+	-	1	2
4	-	+	+	2	3
5	-	-	-	2	2
6	-	+	-	2	1
7	+	-	+	3	2
8	-	+	-	2	3

With regards to the severity, hypocalcemia was mild in 10 patients (54.5%), moderate in 6 patients (33.3%) and severe in 2 patients (11.1%), as shown in Figure 11. None of these patients had chronic hypocalcemia, either clinically overt or biochemically confirmed, 6 months after the procedure.

None of the patients included in the study had symptoms or signs of vocal cord palsy, either transient or permanent.

The procedure of parathyroid autotransplantation was extensively practised by Well SA, Jr., et al., who employed this procedure in the setting of hyperparathyroidism. With time, the application of this procedure in total thyroidectomy was popularized.

In case of thyroidectomy, the objective of performing parathyroid autotransplantation is prevention of permanent hypoparathyroidism, especially in cases of thyroid malignancy involving neck dissection and the inherent risk of parathyroid tissue loss. So, the rate of functionality and viability of the graft will have a bearing on the rate of permanent hypoparathyroidism and chronic hypocalcemia.

Various studies have been conducted to estimate the parathyroid function after parathyroid autotransplantation in thyroid surgery. Largely, indirect evidence of graft function has been reported.

Delbridge, et al. advocates parathyroid autotransplantation as a routine during total thyroidectomy. In their study [55], functionality and viability of the grafts was evident 3 months after total thyroidectomy and parathyroid autotransplantation. While PTH secretion from the grafts was not documented, none of the 100 patients included developed permanent hypoparathyroidism. In another study [58], normocalcemia was observed within 14 weeks of the procedure in all the patients. However, direct assessment of the graft function was not reported. Palazzo, et al. [80]

reported incidence of temporary hypoparathyroidism ranging between 9.8% and 31.4% and that of permanent hypoparathyroidism ranging between 0% and 0.98% after total thyroidectomy and parathyroid autotransplantation. The function of the grafts per se was not reported. In their study, Wells SA Jr., et al. [75] performed parathyroid autotransplantation after total, subtotal or completion thyroidectomy in 104 patients. Successful parathyroid autotransplantation was reported in 103 of these patients. So the rate of functionality was 99% in this study. According to Feldman, et al. [105], nearly 53% of patients who underwent parathyroid autotransplantation had functioning grafts 3 years after the surgery. While the grafts were fully functional in 33% of the patients, partial graft function was demonstrated in 20% of the patients. The grafts were non functional in about 40% of the patients. In another study by Herrera, et al. [104], graft function was evident 5 years after the surgery in over 60% of the patients who underwent parathyroid autotransplantation. In another study [106], 95% of the grafts were reported to be functional after 6 months.

In the present study, the overall rate of graft function, as evidenced by a test-to-control ratio of 1.5 or more, was 72.9% (35 out of the 48 patients who were included in the study).

While the rate of functionality is consistent with that seen in other studies, as mentioned above, the goal of the procedure should be to achieve a 100% functionality rate.

The definitive means of confirmation of parathyroid tissue in the harvested sample is by microscopic examination of frozen sections. Transplantation after confirmation of parathyroid tissue in the sample will ensure that the grafts contain parathyroid tissue.

Frozen section examination of the harvested sample was not performed in the present study.

Wells SA Jr., et al. has recommended that the graft be transplanted within 120 minutes of harvest [75]. In this study, when cold ischemia time duration of the graft was correlated with functionality, a significant difference was observed in the mean cold ischemia time between the viable and the non-viable subsets of patients. Although all grafts were transplanted within 120 minutes of harvest, the harvested bits were stored in sterilized iced saline. However, it was discovered that by placing the harvested glands in iced saline, the grafts were rendered firmer in consistency and so, mincing was easier.

According to Testini, et al., meticulous hemostasis was performed in the recipient pocket to prevent collection of a hematoma or a seroma, in order to avoid potential destruction of graft [24]. So, it is evident that

bleeding in the graft bed, resulting in a hematoma or a seroma, is one of the factors which influence graft survival.

In the present study, diffuse ooze was noted from the subcutaneous and intramuscular planes during dissection at the graft bed in some patients. Diathermy was not used to control this in order to preserve the viability and perfusion of the graft bed. While it was controlled during surgery with digital pressure, it may have led to hematoma or seroma formation in some patients.

Extrusion of the harvested tissue may be another factor which will impact the overall functionality rate. In the present study, the likelihood of graft extrusion is very minimal as the graft was minced in saline and the resultant suspension was injected into the muscle belly.

The pattern of venous drainage of the forearm is another important factor that may influence assessment of graft function. In the present study, the grafts were implanted into the brachioradialis muscle and blood samples were taken from the subcutaneous veins in the cubital fossa. While such veins are invariably present, anatomical variations are extremely common. While the graft may be viable and functional in reality, the assessment may be faulty due to a misrepresentative sample.

Stability of the sample is another important factor. PTH, being a peptide hormone, is labile. It is vital to process the sample at the earliest

while estimating PTH levels. However, degradation of PTH in vitro can be effectively delayed by adding enzyme inhibitors in the tube in which samples are drawn. It has been found that samples collected in such tubes remain stable for a couple of hours or so.

In the present study, such tubes with enzyme inhibitors were indeed employed.

Various other factors may influence PTH gradients, leading to confounding results, although the patients have viable grafts. PTH secretion itself may be intermittent. The iPTH gradient may be influenced by the regional specificity of the iPTH assay employed.

In another study, there was no correlation between the test-to-control ratio of PTH levels and the level of overall graft function [103]. In some patients with partial graft function and PTH levels in the normal range, hypocalcemia occurred on cessation of calcium supplementation.

In another study [104], patients with minimal PTH gradients were found to be eucalcemic with sufficient graft function after total parathyroidectomy and parathyroid autotransplantation. Some others with significant gradients had clinically overt hypoparathyroidism.

Transient hypoparathyroidism after thyroidectomy with parathyroid autotransplantation has been widely reported [93-99]. While the protective role of parathyroid autotransplantation in definitive

hypoparathyroidism has been generally accepted, the effect on transient hypo-parathyroidism is still debated.

It has been reported that inadvertent parathyroidectomy during thyroidectomy does not cause postoperative transient hypocalcemia [100, 101]. Testini, et al. [24] reported that parathyroid autotransplantation was protective against transient hypoparathyroidism. The rates of transient hypocalcemia were 0% and 2.5% respectively, in those who underwent parathyroid autotransplantation along with total thyroidectomy and in those who underwent total thyroidectomy alone. According to Karakas, et al. [105], the incidence of transient as well as permanent hypocalcemia was significantly less in those who underwent parathyroid autotransplantation, when compared with those who did not.

There may be a transient release of PTH immediately following parathyroid autotransplantation. Although the transplanted glands seem to start functioning after a minimum of 2 weeks, high levels of PTH in the minced tissue have been shown [102].

Nevertheless, it is also believed that parathyroid autotransplants are unlikely to restore eucalcemia in the immediate postoperative period.

The rate of permanent hypoparathyroidism is not a product of the number of glands transplanted at the time of surgery [80].

In the present study, among the patients who were analyzed, post-operative hypocalcemia was seen in 18 patients (37.5%). This was not significantly different from the rate of post-operative hypocalcemia in other studies involving total thyroidectomy alone. In addition, none of the patients who were operated had hypocalcemia or hypoparathyroidism 6 months after the procedure.

One particular patient who was included in this study merits mention due to the unusual pattern of results post-operatively. The patient was operated for papillary thyroid carcinoma. Total thyroidectomy with central compartment lymph node dissection and parathyroid autotransplantation was performed. When the graft function was analyzed after 90 days, the graft was found to be viable and functional, with a test-to-control ratio well above 1.5. However, when a whole body radioiodine scan was performed as part of the post-operative follow-up for PTC, a hot spot was evident in near the graft bed.

In summary, while the rate of functionality of the parathyroid grafts after autotransplantation was 72.9%, the grafts in the remaining patients might be non-functional according to this study due to the following causes – absence of parathyroid tissue in the harvested sample, prolonged cold ischemia time, seroma or hematoma formation in the graft

bed, misrepresentative samples due to anatomical variations in the pattern of venous drainage of the forearm and issues with sample stability.

In the present study, the overall rate of functionality of the parathyroid grafts after total thyroidectomy and parathyroid autotransplantation was 72.9%. Six months after autotransplantation into the brachioradialis, grafts were functioning in 35 out of 48 patients.

The rate of functionality after autotransplantation was significantly influenced by cold ischemia time of the harvested gland. Transplantation immediately after harvesting, before proceeding with further dissection and surgery, may yield higher functionality rates.

Graft survival was not significantly influenced by the other factors evaluated, including thyroid pathology or the presence of thyroiditis. With regards to the site of the harvested gland, the functionality rate was apparently higher when the superior parathyroids were harvested, although this difference was not statistically significant.

In this study, the overall rate of transient post-operative hypocalcemia was 37.5%. None of the patients included in this study developed permanent post-operative hypoparathyroidism.

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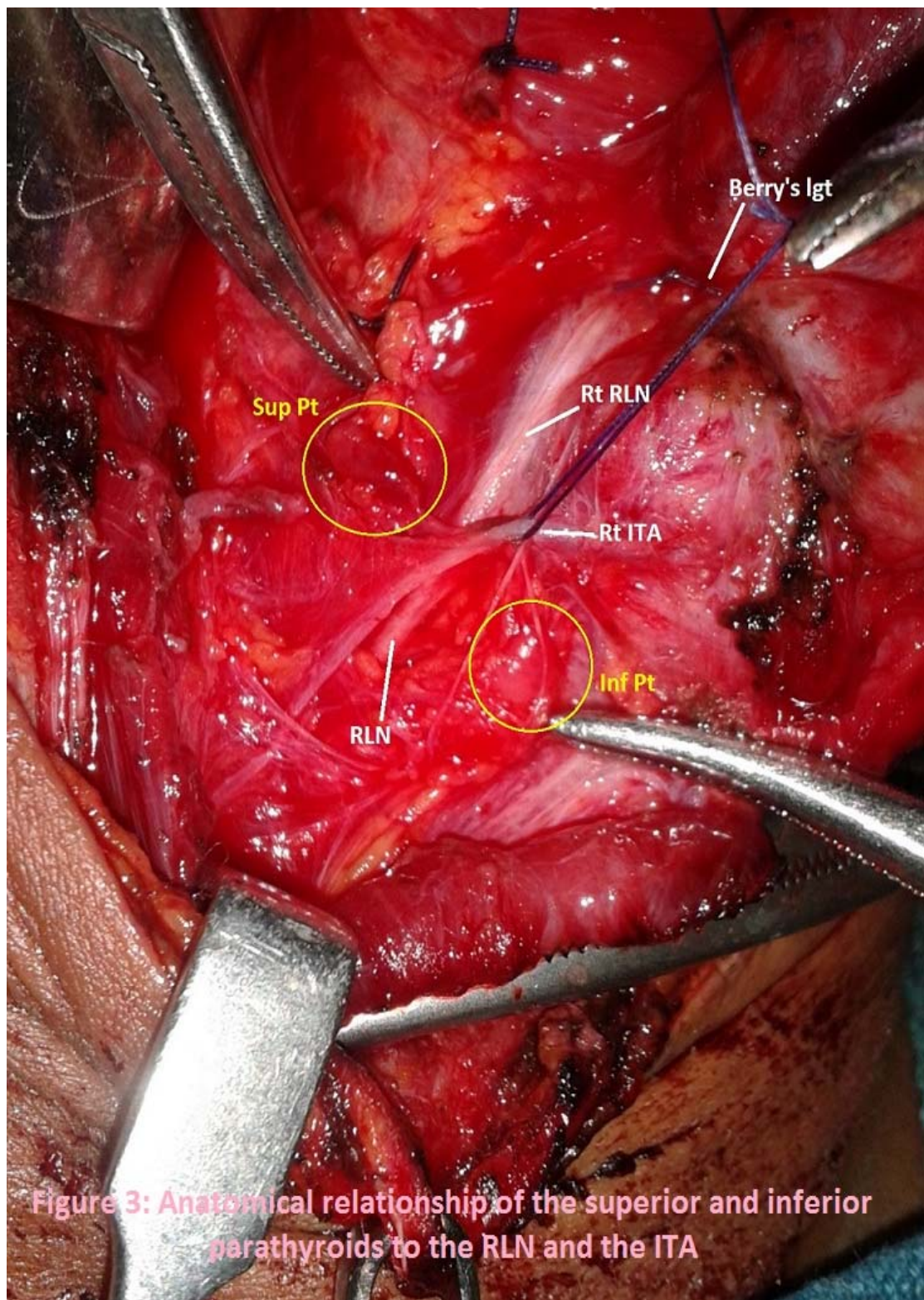


Figure 7: S. PTH levels in the test and control arms of patients

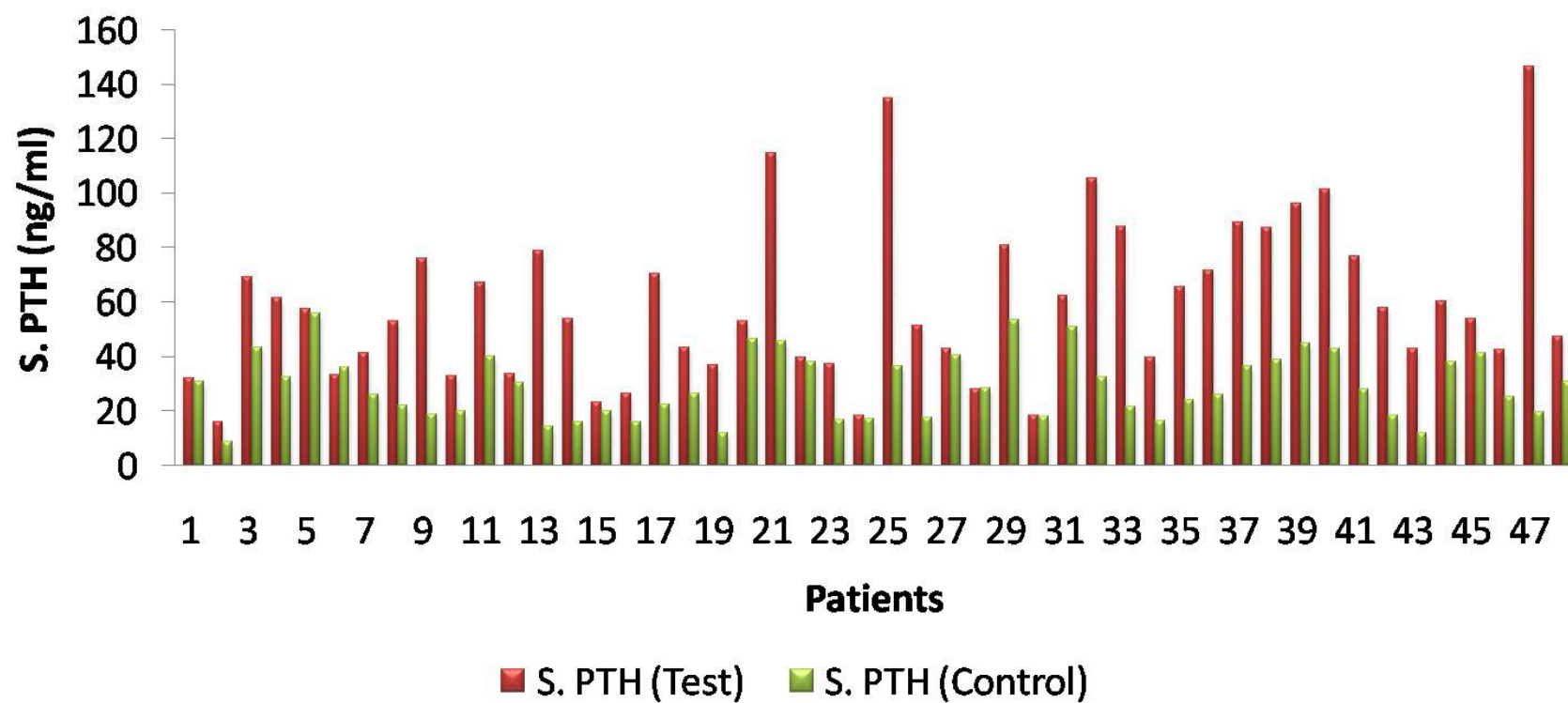


Figure 8: Test-to-Control ratios in all patients

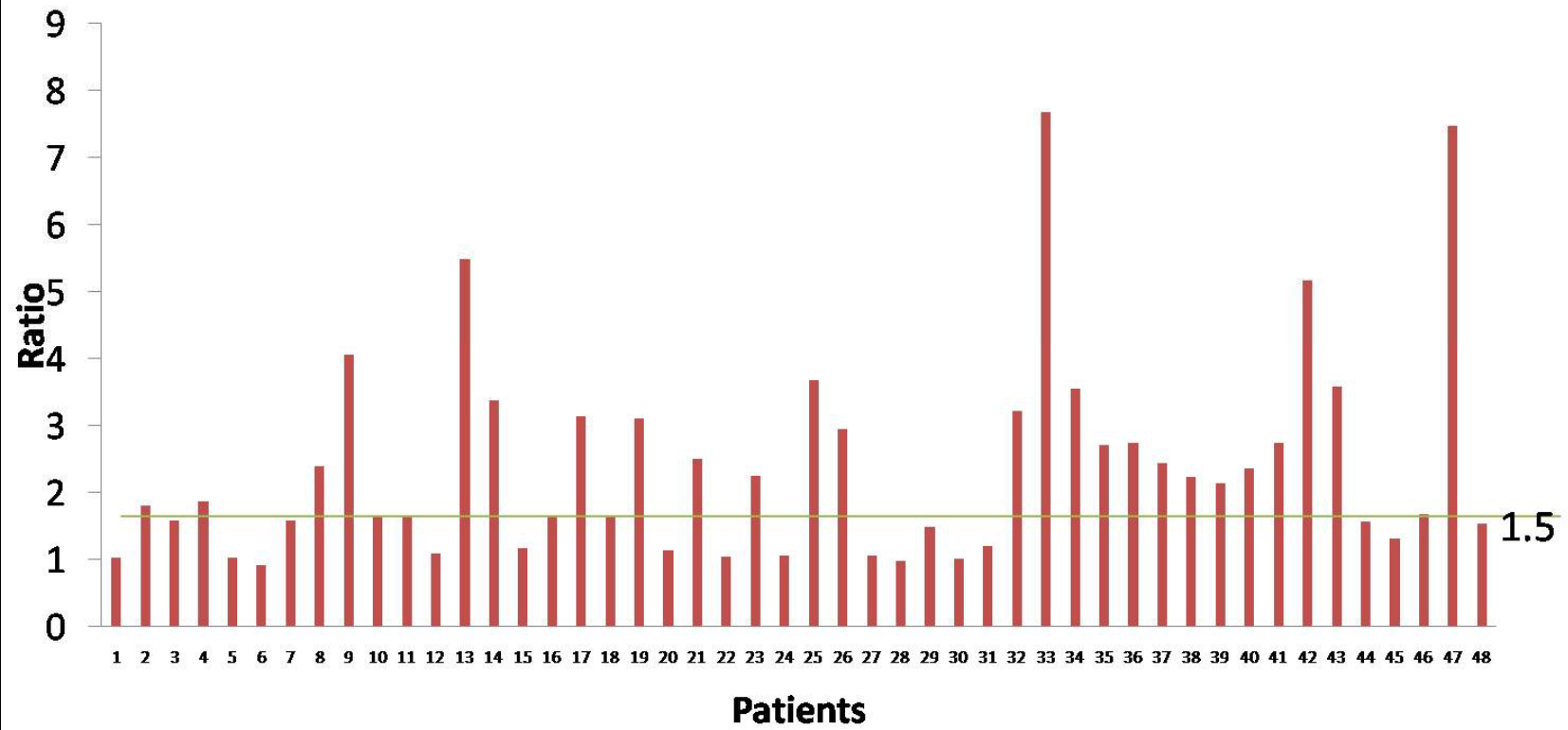


Figure 9: Cold ischemia times for viable grafts

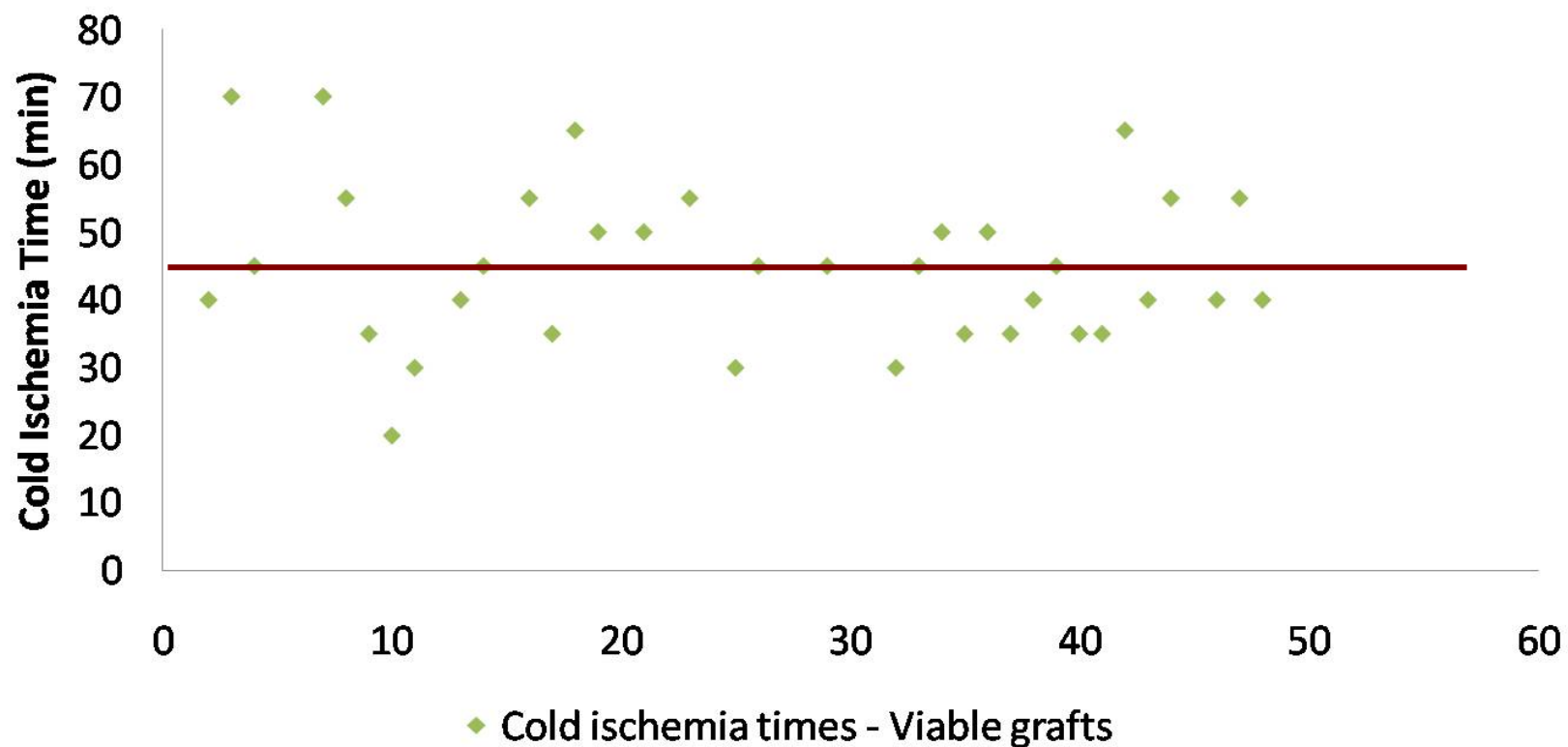


Figure 10: Cold ischemia times for nonviable grafts

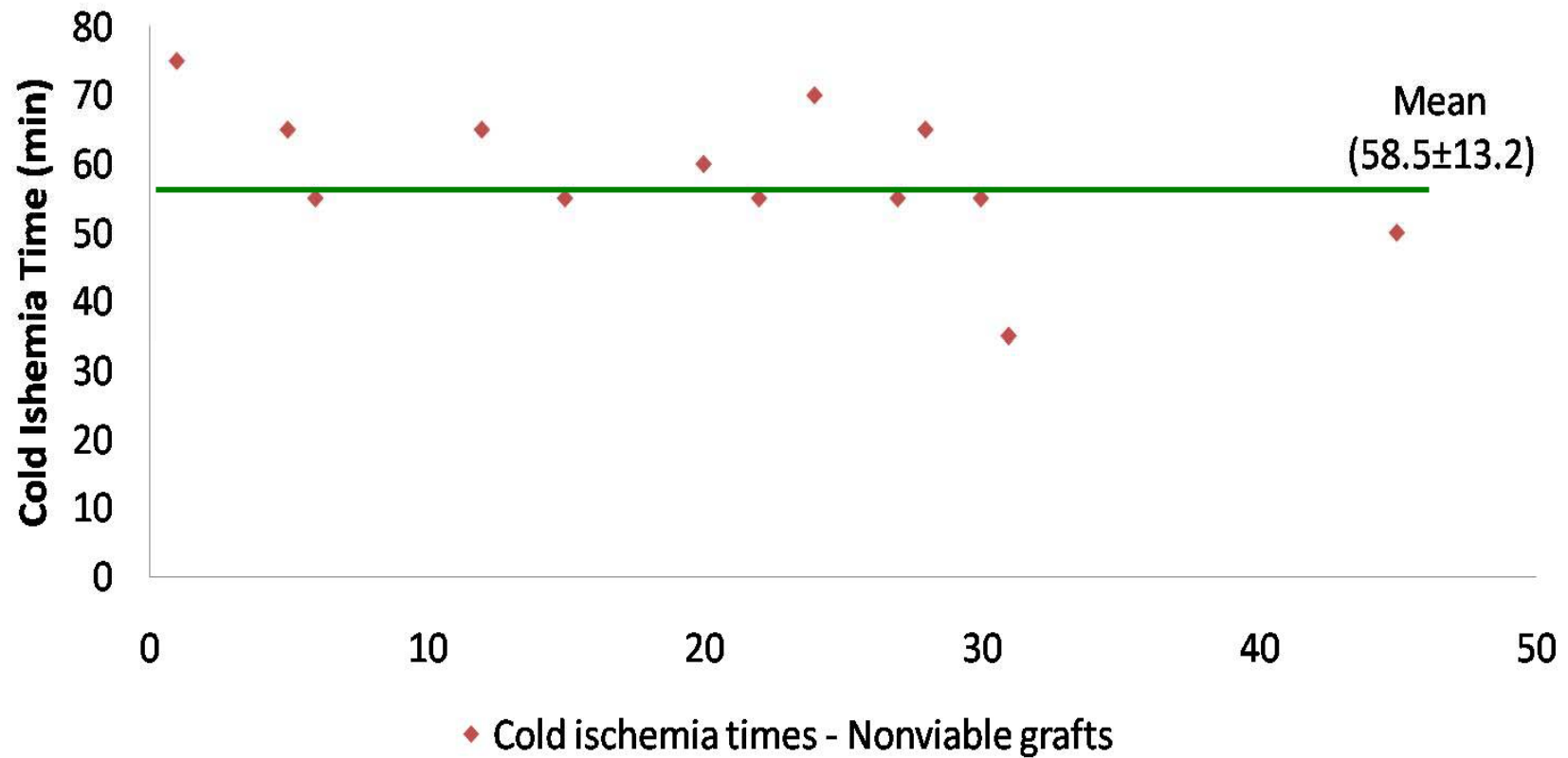
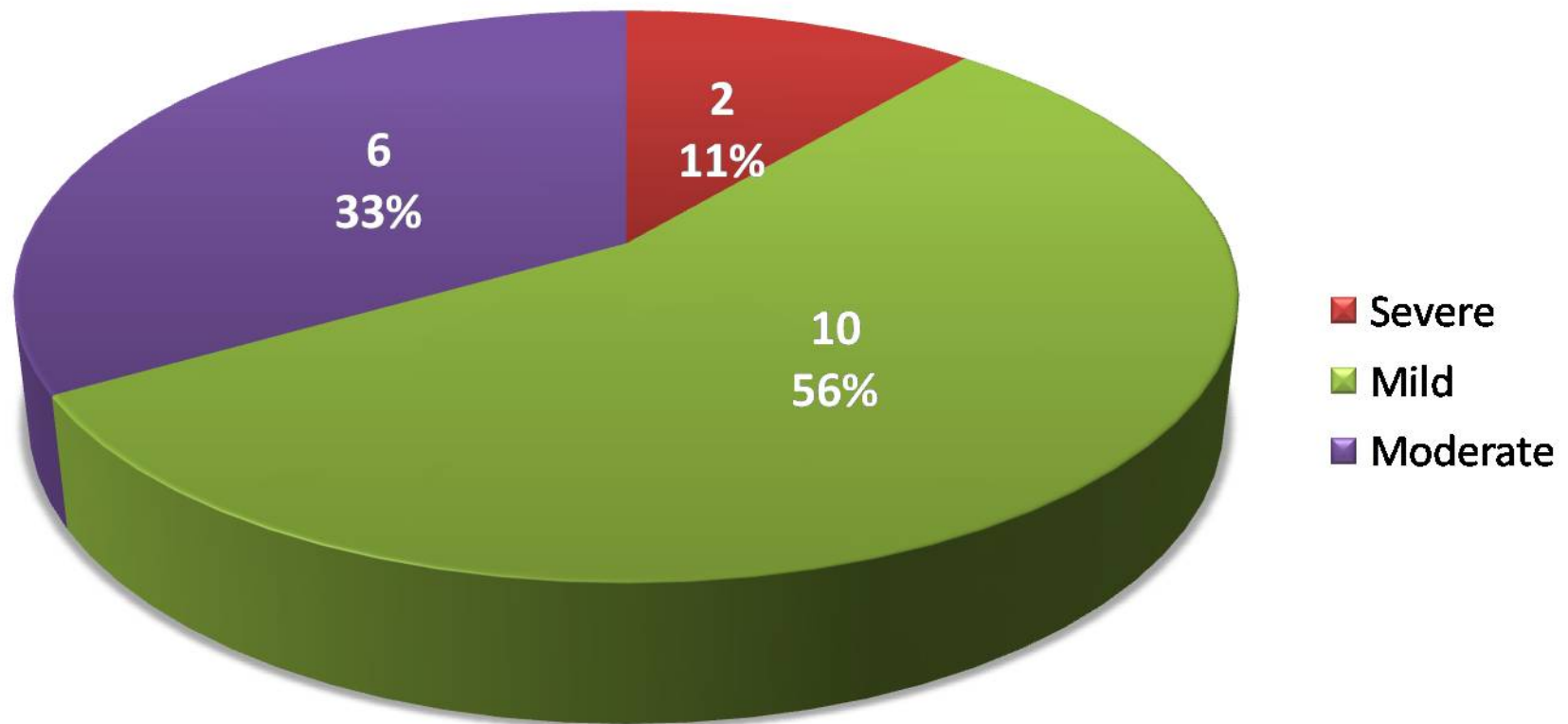


Figure 11: Degree of hypocalcemia - frequency



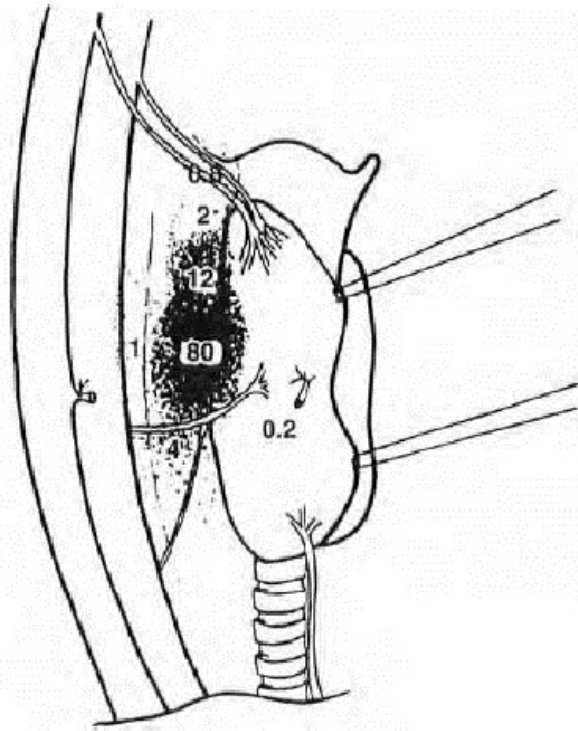


Figure 1: Distribution of superiro parathyroids in the body

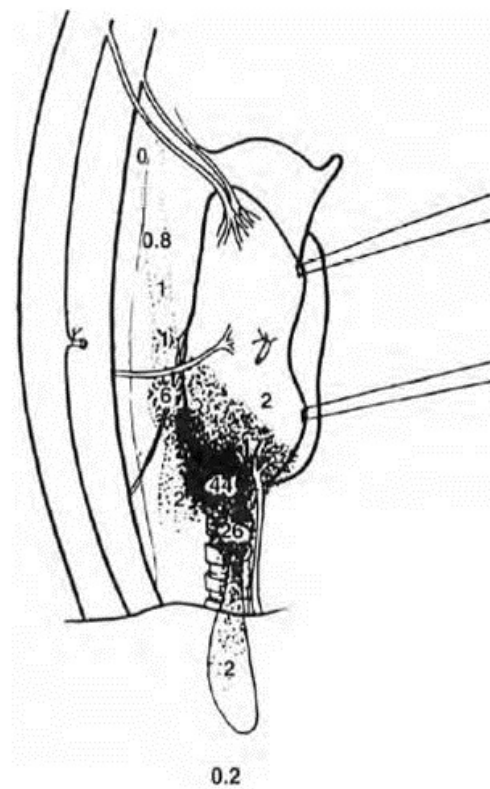


Figure 2: Distribution of inferiro parathyroids in the body



Figure 4: Mincing of the harvested gland in iced saline



Figure 5: Exposure of the brachioradialis muscle

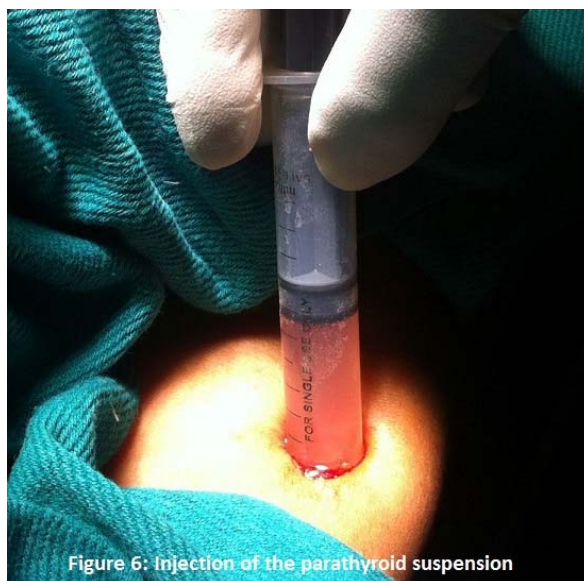
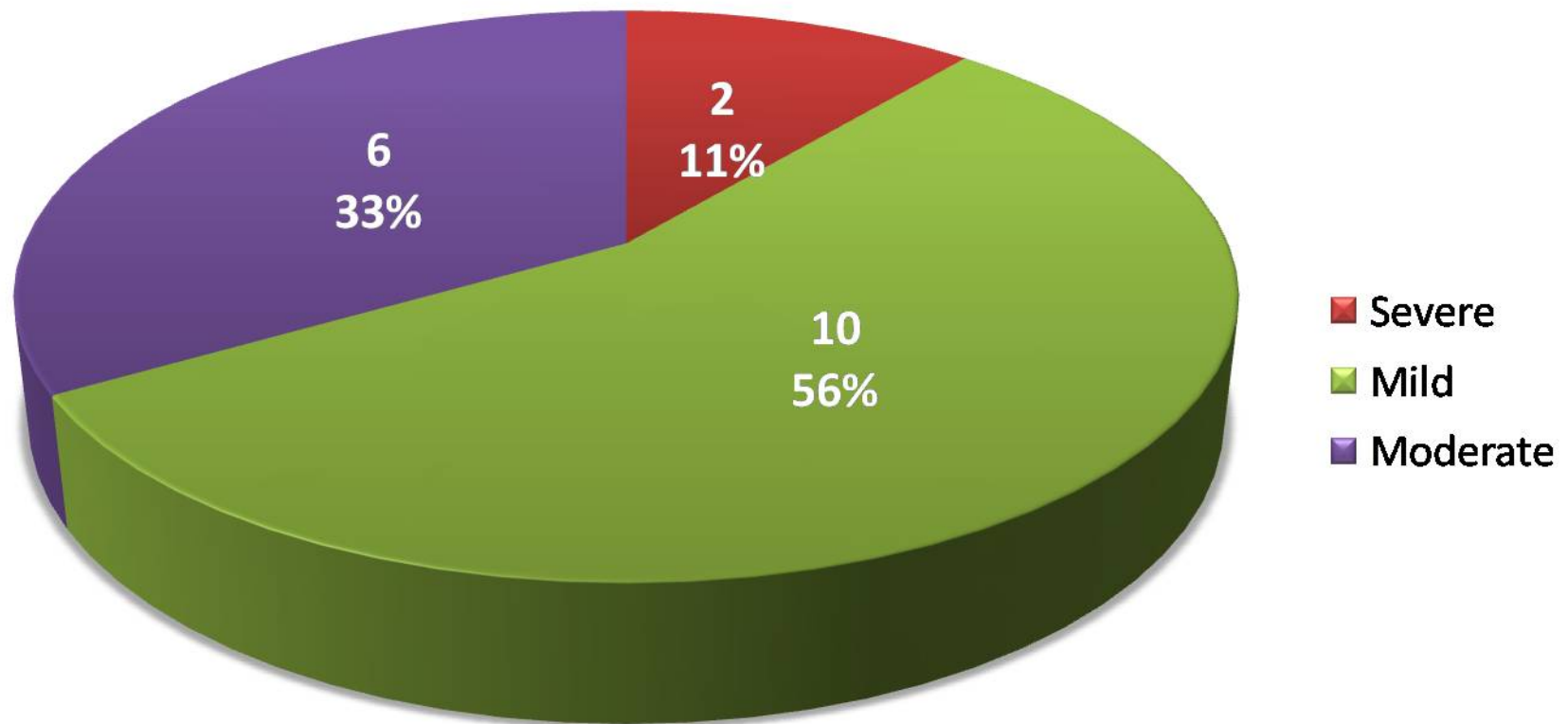


Figure 6: Injection of the parathyroid suspension

Figure 11: Degree of hypocalcemia - frequency



INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No : 044 25305301

Fax : 044 25363970

CERTIFICATE OF APPROVAL

To

Dr. Himagirish K.Rao
PG in MCH Endocrine surgery
Madras Medical College, Chennai -3

Dear Dr. Himagirish K.Rao

The Institutional Ethics committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled "Assessment of functional status of auto-transplanted parathyroid glands after total thyroidectomy" No. 18112011

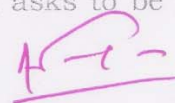
The following members of Ethics Committee were present in the meeting held on 22.11.2011 conducted at Madras Medical College, Chennai -3.

- | | |
|--|---------------------|
| 1. Prof. S.K. Rajan. MD | -- Chairperson |
| 2. Prof.A. Sundaram MD
Vice principal, Madras Medical College, Ch -3 | -- Member Secretary |
| 3. Prof. R. Nandhini MD
Director, Institute of Pharmacology ,MMC, Ch-3 | -- Member |
| 4. Prof. Pregna B. Dolia MD
Director , Institute of Biochemistry, MMC, Ch-3 | -- Member |
| 5. Prof. C. Rajendiran, MD
Director , Inst. Of Internal Medicine, MMC, Ch-3 | -- Member |
| 6. Prof. Md Ali MDDM
Prof & Head , Dept. of MGE, MMC,Ch-3 | -- Member |
| 7. Prof. Shantha Ravishankar MD
Prof of Neuropathology, MMC, Ch-3 | -- Member |
| 8. Thiru. S. Govindsamy. BA BL | -- Lawyer |
| 9. Tmt. Arnold soulina MA | -- Social Scientist |

We approve the proposal to be conducted in its presented form.

Sd/ chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.



Member Secretary, Ethics Committee